Selegiline in the treatment of Alzheimer's disease: a long-term randomized placebo-controlled trial

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Objective: To evaluate the efficacy and adverse effects of the type B monoamine oxidase inhibitor selegiline (also known as l-deprenyl) in the treatment of Alzheimer's disease. **Design:** Long-term, double-blind, placebo-controlled trial. **Setting:** Seven cities (1 or 2 nursing homes in each city) in the Czech and Slovak Republics. **Patients:** A total of 173 nursing-home residents fulfilling the DSM-III criteria for mild to moderate Alzheimer's disease. **Interventions:** Selegiline (10 mg per day) or placebo (both including 50 mg ascorbic acid) administered for 24 weeks. **Outcome measures:** Clinical Global Impressions scale and Nurses Observation Scale for Inpatient Evaluation at baseline and at weeks 6, 12 and 24; Clock Drawing Test at baseline and 24 weeks, results of which were evaluated as normal or pathologic, and quantitatively on a modified 6-point scale; Sternberg's Memory Scanning test at baseline and at weeks 6, 12 and 24; Mini Mental State Examination, and electroencephalogram at baseline and 24 weeks; Structured Adverse Effects Rating Scale; physical, laboratory, hematological and electrocardiographic examinations at baseline and weeks 12 and 24. **Results:** A total of 143 subjects completed enough of the trial to be entered in the analysis. Subjects were analyzed by 2 subgroups depending on whether they had a normal or pathologic result of the Clock Drawing Test. Analysis of variance showed significant improvement with selegiline versus placebo among those with a normal result of the Clock Drawing Test on the Mini Mental Status Examination (total score and orientation-place subscale) and among those with a pathologic result of the Clock Drawing Test on Sternberg's Memory Scanning test (for both speed and accuracy), on the Clinical

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Global Impressions scale as well as in terms of the dominant frequency on electroencephalograms. **Conclusion:** Selegiline has a long-term beneficial effect in Alzheimer's disease on memory modalities that reflect the function of the prefrontal areas of the brain, which are rich in dopamine receptors. The delayed appearance of differences between selegiline and placebo supports the notion that the mechanism of action is through neuronal rescue or neuroprotection. The differential response of patients with normal and pathologic results of the Clock Drawing Test may reflect the fact that the evaluation methods' sensitivity to change depends on the severity of dementia.

Objectif : Évaluer l'efficacité et les effets indésirables de la sélégiline (aussi appelée l-deprenyl), inhibiteur de la monoamine oxydase de type B dans le traitement de la maladie d'Alzheimer. **Conception :** Étude de longue durée, à double insu et contrôlée par placebo. **Contexte :** Sept villes (un ou deux foyers de soins infirmiers dans chaque ville) dans les républiques tchèque et slovaque. **Patients :** Au total, 173 résidents de foyers de soins qui satisfaisaient aux critères DSM-III portant sur la maladie d'Alzheimer bénigne à moyenne. **Interventions :** Administration de sélégiline (10 mg par jour) ou d'un placebo (les deux contenant 50 mg d'acide ascorbique) pendant 24 semaines. **Mesures de résultats :** Échelle des impressions cliniques globales et échelle des observations infirmières pour l'évaluation de patients hospitalisés, au niveau de référence et à 6, 12 et 24 semaines; test du cadran au niveau de référence et à 24 semaines, dont les résultats ont été jugés normaux ou pathologiques et évalués quantitativement sur une échelle modifiée à six points; test de mémoire de Sternberg au niveau de référence et à 6, 12 et 24 semaines; mini-examen de l'état mental et électroencéphalogramme au niveau de référence et à 24 semaines; échelle structurée d'évaluation des effets indésirables; examen médical, tests de laboratoire et d'hématologie, et électrocardiographie au niveau de référence et à 12 et 24 semaines. **Résultats :** Au total, 143 sujets ont terminé une partie suffisante de l'étude pour participer à l'analyse. On a analysé les sujets répartis en deux sous-groupes selon que le test du cadran donnait un résultat normal ou pathologique. L'analyse des écarts a montré une amélioration importante avec la sélégiline plutôt qu'avec le placebo chez ceux qui ont obtenu un résultat normal au test du cadran et au mini-examen de l'état mental (résultat total et sous-échelle orientation-lieu) et chez ceux qui ont obtenu un résultat pathologique au test du cadran, au test de mémoire de Sternberg (vitesse et exactitude), à l'échelle des impressions cliniques globales et en ce qui concerne la fréquence dominante des électroencéphalogrammes. **Conclusion :** Dans les cas de maladie d'Alzheimer, la sélégiline a un effet bénéfique de longue durée sur les modalités de la mémoire qui reflètent le fonctionnement des zones préfrontales du cerveau, riches en récepteurs de la dopamine. L'apparition retardée de différences entre la sélégiline et le placebo appuie le concept selon lequel le médicament agit par sauvetage de neurones ou neuroprotection. La réponse différentielle des patients qui ont obtenu des résultats normaux et des résultats pathologiques au test du cadran peut refléter le fait que la sensibilité au changement des méthodes d'évaluation dépend de la gravité de la démence.

Functional impairment of several neurotransmitter systems, especially those regulating acetylcholine, dopamine and serotonin, plays an important role in the pathogenesis of Alzheimer's disease (AD).[1] The dysfunction of the dopaminergic system is associated with an increase in the activity of type B monoamine oxidase (MAO-B), the enzyme involved in the degradation of dopamine. This increase is apparent in platelets and in the brain tissue of patients with AD, and correlates with the severity of dementia.[2]

Selegiline (also known as l-deprenyl) is an irreversible inhibitor of MAO-B. In humans, it is specific for type B MAO in dosages of less than 10 mg per day.[3] In addition, selegiline may act as an antioxidant in neurons[4–6] and protect against glutamate-receptor-mediated toxicity.[7]

Another putative mechanism of action, independent of MAO inhibition, is rescue of damaged neurons.[8] The rescue mechanisms may involve stimulation of neurite outgrowth,[9] stimulation of gene expression in preapoptotic neurons,[10] or stimulation of cytokine biosynthesis.[11]

Administering selegiline to aged male laboratory animals slowed their cognitive and behavioural deterioration and significantly prolonged their average life span in comparison with control animals.[4,12]

Martini et al[13] were the first to notice, in an open trial, the beneficial effects of selegiline in Alzheimer's disease. The results of short-term (up to 3 months) double-blind placebo-controlled trials confirmed this initial observation.[14–18] Two long-term trials yielded a negative result concerning the therapeutic efficacy of selegiline; however, they both suffered from a small sample size.[19,20] In contrast, a 2-year placebo-controlled trial by Sano et al,[21] in which life events were the primary response variable, demonstrated a slowing of progression of Alzheimer's disease in patients treated with selegiline and with α-tocopherol, a potent antioxidant.

We designed a long-term, phase III, double-blind, placebo-controlled, randomized, multicentre therapeutic trial to investigate whether long-term administration of low-dose selegiline to patients in the early stage of AD would partly reverse mental changes related to dementia, or slow their progression.

The study consisted of a pretreatment phase, lasting 4 to 8 weeks, followed by a postrandomization double-blind treatment phase of 6 months' duration, and an open follow-up phase of 6 months' duration. This report covers only the double-blind phase of the trial.

Methods

Subjects

The participating centres were located in 7 cities (Table 1). Each centre recruited the patients in 1 or 2 nursing homes. The patients were screened and then followed by a team consisting of a psychiatrist, a psychologist and a general practitioner familiar with the patient's medical history. Taking into consideration that the assessment methods' sensitivity to change may depend on the severity of the disease,[22] we used the Clock Drawing Test[23] to classify disease severity as normal or pathologic, which allowed us to focus on patients subgroups.

The study population consisted of 173 male and female residents of nursing homes, who met the following entry criteria: (1) age 60 years or older, (2) diagnosis of uncomplicated, late-onset, primary degenerative dementia (code 290.00 in the *Diagnostic and Statistical Manual of Mental Disorders, third edition*), of mild to moderate severity, (3) Mini Mental State Examination score of 24 or lower, (4) Hachinski Ischemic Score[24] of 0 to 4, (5) informed consent, (6) no other major psychiatric or physical disorder, (7) no substance abuse, and (8) ability to complete the psychometric tests. A computed tomographic scan was taken to detect organic brain lesions other than Alzheimer's disease (e.g., infarcts or tumours).

Medication

We used an active placebo in the form of low-dose ascorbic acid. To balance any effects of ascorbic acid, it was administered to the selegiline group as well. The aim was to increase the resistance of the study population against common viral diseases.

Hence, subjects were administered gelatine capsules containing either 10 mg of lactose and 50 mg of ascorbic acid or 10 mg of selegiline and 50 mg of ascorbic acid.

The experimental medication was administered according to a fixed dosage schedule of 1 capsule once a day in the morning hours. During the pretreatment phase, all patients received the placebo capsules for at least 4 weeks before the baseline psychometric examination.

Patients already being treated with drugs influencing the central nervous system were not included in the trial; however, if necessary, the administration of centrally active compounds was allowed provided it did not exceed a cumulative period of 4 weeks.

Assessment of change

All investigators participated in a 3-day training session in diagnostic procedures and administration of the behavioural and cognitive assessment instruments before the trial began.

The patients underwent behavioural and cognitive assessment at baseline and then at weeks 6, 12 and 24; assessment instruments that were not available in mul-

Table 1: Demographic parameters of patients with Alzheimer's disease randomly assigned to selegiline or placebo

Centre	Number of patients	Average age, yr	% with pathologic results of Clock Drawing Test	Average score on Mini Mental State Examination
Bratislava	26	82	77	18
Brno	26	85	81	18
Olomouc	26	82	15	21
Hradec Králové	24	80	75	20
Plzen	26	83	50	17
Mladá Boleslav	22	82	86	19
Praha	23	85	52	19
Total	173	83	62	19

tiple equivalent versions were used at baseline and week 24 only.

To assess behaviour, we used the Clinical Global Impressions (CGI) scale[25] and the Nurses Observation Scale for Inpatient Evaluation (NOSIE).[26] The evaluation of CGI was based mainly on the report of the nursing staff, who were in everyday contact with the patients but did not know the results of the performance tests.

To assess cognitive function, subjects were asked to draw a clock at baseline and after 24 weeks using the procedure described by Wolf-Klein et al.[23] The pictures thus generated were evaluated by 2 methods serving different purposes. First, the clock drawing pattern was evaluated as either normal or pathologic (Clock Drawing Test[23]). This evaluation served to identify disease stage (early versus advanced), and was used as a grouping factor in the statistical analysis. Second, the clock drawing pattern was evaluated quantitatively on a modified 6-point scale (Clock Drawing Scale[27]), as one of the measures of cognitive change.

Sternberg's Memory Scanning[28,29] test was selected as the primary end point. This is a neuropsychological method allowing differentiation of the peripheral (speed of sensorimotor processing) and central (speed of memory scanning) composition of reaction time during a recognition task. In our modification, each examination consisted of 3 tasks with increasing complexity. The tasks, administered consecutively, consisted of the recognition of 1, next 2, and finally 4 target photographs of human faces among 25 photographs (12 target photographs and 13 distractors), which were presented after the respective target photographs had been successfully memorized. The first observed variable in each respective task was the total response time (the time needed to check all 25 photographs). The second observed variable was the error rate. The target size was then plotted against the response time of the respective series, and the intercept and slope of each individual curve was calculated. The test was available in 3 equivalent versions.

The Mini Mental State Examination[30] was conducted at baseline and after 24 weeks.

An electroencephalogram was recorded on paper with a standardized montage at pretreatment and after 24 weeks. The mean frequency of α and other dominant frequencies was evaluated visually (in 0.5-Hz steps) by a single rater using randomized, blinded sequence of baseline and final recordings.

For safety monitoring, the Structured Adverse Effects Rating Scale (SARS)[31] was used. The patients underwent physical, laboratory, hematological and electrocardiographic examinations at pretreatment, after 3 months and at termination.

Statistical analysis

The principal method of statistical evaluation was analysis of variance (ANOVA) with 2 grouping variables: the drug administered (MEDICATION: selegiline or placebo) and the result of the Clock Drawing Test (CLOCK: normal or pathologic), and one repeating variable, the period of the testing (PERIOD: baseline, week 6, week 12 and week 24). To ascertain the time points at which there were significant differences between the 2 treatment groups, the model was expanded by analysis of contrasts of the particular assessment periods against baseline, including the interaction of the contrast with the drug administered. Any deviations from the model described are specified below in Results.

If the basic ANOVA model indicated a triple interaction of the drug administered, the result of the Clock Drawing Test and the period of the testing (MEDICATION $\times$ CLOCK $\times$ PERIOD), a reduced ANOVA model, without CLOCK, was applied for each of the Clock Drawing Test subgroups (normal or pathologic) separately, to evaluate in which subgroup the interaction between the drug administered and the period of testing (MEDICATION $\times$ PERIOD), indicating a difference between active drug and placebo, was significant.

For analysis of the categorical data (death rate and CGI score), nonparametric tests (χ^2 and Mann-Whitney U test, respectively) were used.

Results were considered statistically significant at a p value of 0.05 or less. The primary end point was the result of Sternberg's Memory Scanning test. Other variables were considered secondary end points and their analysis had an exploratory character; therefore, the conservative procedures of simultaneous statistical inference were not implemented.

BMDP statistical software (Statistical Solutions, Cork, Ireland) was used for all statistical computations.

Results

The basic demographic parameters of the randomized sample, by centre, are shown in Table 1.

Of 173 study participants who entered the postrandomization phase, 113 underwent the computed tomographic (CT) examination. The remaining 60 subjects could not be examined, mainly because they refused to travel to the hospital. In these patients, other causes of dementia were excluded by detailed case history, Hachinski score, and clinical and laboratory (thyroxine level) examination.

The CT examinations revealed 24 single and 15 multiple (2 or more) cerebral infarcts, ranging in volume from 0.5 to 53.6 mL (median 10.8 mL). Since vascular lesions with a total volume lower than 50 to 100 mL are unlikely to cause dementia,[32,33] no patients were excluded on basis of the CT examination.

One hundred and forty-two patients completed the 6-month trial according to the experimental protocol (Table 2). One subject was withdrawn on day 163, but most of his cognitive and behavioural testing had been completed. Therefore, the computations were based on 143 individuals unless there was missing data (indicated in the tables).

Table 3 shows the reasons for subject drop-out. There were 6 deaths in the selegiline group (bronchopneumonia in 3 patients, and hip fracture, prostate cancer and cerebral stroke in 1 patient each), and 4 in the placebo group (heart attack, suicide, nonspecified internal bleeding and rupture of esophageal veins in 1 patient each). No relation between these events and treatment was found. It is noteworthy that in 7 out of 9 patients who dropped out of the selegiline group by week 13, the reason given was uncooperativeness or restlessness. No such reason for drop-out was given in the placebo group.

Ten subjects had low plasma levels of vitamin B_{12} (< 120 pg/mL); no abnormal thyroxine levels were found.

Efficacy

Evaluation of any significant difference between the treatment groups was based on the effect of the interactions between drug administered and period (variables MEDICATION and PERIOD) or drug administered and contrast (variables MEDICATION and CONTRAST) in the ANOVA model.

We found no significant difference in the severity of illness between the treatment groups according to the CGI (MEDICATION × PERIOD $F[df\,2, 226] = 0.58$, $p = 0.559$ and MEDICATION × PERIOD × CLOCK, $F[df\,2, 226] = 2.25$, $p = 0.108$).

Also according to the CGI, after 6 months, the patients with a normal result of the Clock Drawing Test were rated on average as minimally improved in both treatment groups. The average rating of the patients with a pathologic result of the test was "minimally improved" in the selegiline group and "unchanged" in the placebo group (Mann-Whitney U, $p < 0.005$).

According to the NOSIE, a temporary increase in irritability was observed after 6 weeks of therapy with selegiline. This was the reason for drop-out among some subjects (Table 3).

In terms of cognitive functioning, Table 4 shows the results of Sternberg's Memory Scanning test. In the subgroup of patients with pathologic results of the Clock Drawing Test, there was a slight increase in the sensorimotor component of the reaction time in the placebo group, as measured by the intercept of the reaction time curve, whereas there was a marked decrease in the intercept in the selegiline group (MEDICATION × PERIOD, $p = 0.002$). The analysis of the time contrasts

Table 3: Reasons for early termination of participation in clinical trial of selegiline treatment of Alzheimer's disease

	Group, no. of patients	
Reason	Placebo	Selegiline
Somatic illness	5	2
Psychosis	2	1
Uncooperativeness	1	7
Death	4	6
Other	2	1
Total	14	17

Table 2: Selected demographic data for the patients who finished the 6-month trial according to the experimental protocol

				Treatment group, no. of patients		Sex, no. of patients	
Subgroup, result of Clock Drawing Test	No. (and %) of patients	Mean age (and standard deviation), yr	Mean score on Mini Mental State Examination (and SD)	Selegiline	Placebo	Male	Female
Normal	51 (36)	82.9 (6.2)	19.7 (3.0)	24	27	20	31
Pathologic	91 (64)	83.2 (7.6)	18.4 (3.8)	47	44	21	70
Total	142 (100)	83.0 (6.7)	18.9 (3.6)	71	71	41	101

Table 4: Results of Sternberg's Memory Scanning test (recognition of faces) — intercept, slope and error rate

				Mean seconds (and standard deviation)				ANOVA						
									Normal results of CDT*			Pathologic results of CDT		
Variable	Group	Subgroup by result of CDT	No. of patients	Baseline	Week 6	Week 12	Week 24	Variable	Degrees of freedom	F	p value	Degrees of freedom	F	p value
Intercept	Placebo	Normal	28	98.3 (53.0)	94.5 (65.4)	86.7 (64.7)	73.5 (55.7)	MEDICATION	1, 48	0.05	0.831	1, 80	0.00	0.987
		Pathologic	36	79.6 (58.3)	74.9 (52.6)	78.3 (49.3)	81.1 (53.5)	PERIOD	3, 144	5.75	0.001	3, 240	4.21	0.006
		Total	64	87.8 (56.4)	83.5 (58.9)	82.0 (56.2)	77.8 (54.2)	MEDICATION ×PERIOD	3, 144	0.70	0.551	3, 240	5.09	0.002
	Selegiline	Normal	22	103.6 (53.4)	95.1 (80.0)	70.4 (34.9)	71.8 (65.2)	WEEK 6	1, 48	0.07	0.797	1, 80	0.30	0.585
		Pathologic	46	94.7 (55.8)	84.4 (51.7)	66.1 (45.6)	68.0 (44.3)	WEEK 12	1, 48	1.68	0.202	1, 80	6.75	0.011
		Total	68	97.6 (54.8)	87.9 (61.9)	67.5 (42.2)	69.2 (51.5)	WEEK 24	1, 48	0.16	0.689	1, 80	6.83	0.011
Slope	Placebo	Normal	28	6.3 (12.8)	4.4 (10.5)	4.9 (12.7)	5.8 (10.3)	MEDICATION	1, 48	0.94	0.337	1, 80	0.22	0.638
		Pathologic	36	6.1 (14.7)	6.5 (7.7)	4.7 (5.7)	3.7 (6.4)	PERIOD	3, 144	0.86	0.465	3, 240	0.87	0.458
		Total	64	6.2 (13.8)	5.6 (9.0)	4.8 (9.3)	4.6 (8.3)	MEDICATION ×PERIOD	3, 144	1.18	0.318	3, 240	2.70	0.047
	Selegiline	Normal	22	4.2 (8.0)	6.2 (15.0)	9.8 (10.6)	9.7 (11.6)	WEEK 6	1, 48	1.10	0.300	1, 80	1.06	0.307
		Pathologic	46	1.7 (11.9)	4.9 (8.3)	6.2 (7.0)	5.6 (8.1)	WEEK 12	1, 48	3.48	0.068	1, 80	3.68	0.059
		Total	68	2.5 (10.8)	5.3 (10.8)	7.4 (8.4)	6.9 (9.5)	WEEK 24	1, 48	2.73	0.105	1, 80	3.71	0.058
Total errors	Placebo	Normal	28	6.6 (3.9)	7.9 (4.9)	9.8 (6.4)	9.5 (7.1)	MEDICATION	1, 48	1.29	0.261	1, 79	0.00	0.973
		Pathologic	35	8.0 (6.8)	8.2 (7.7)	12.1 (10.2)	12.8 (11.8)	PERIOD	3, 144	4.09	0.008	3, 237	7.02	< 0.001
		Total	63	7.4 (5.7)	8.1 (6.6)	11.1 (8.7)	11.3 (10.1)	MEDICATION ×PERIOD	3, 144	1.59	0.194	3, 237	3.06	0.029
	Selegiline	Normal	22	5.8 (5.1)	7.5 (6.1)	8.2 (7.8)	5.9 (7.3)	WEEK 6	1, 48	0.09	0.767	1, 79	0.05	0.817
		Pathologic	46	9.5 (7.2)	10.0 (6.6)	10.5 (7.7)	10.8 (8.6)	WEEK 12	1, 48	0.19	0.668	1, 79	3.84	0.054
		Total	68	8.3 (6.8)	9.2 (6.5)	9.8 (7.8)	9.2 (8.5)	WEEK 24	1, 48	2.67	0.109	1, 79	3.78	0.056

*CDT = Clock Drawing Test.

revealed that the difference between the treatment groups was significant after 12 and 24 weeks of the treatment (p = 0.011 and 0.011, respectively). In the patients with normal results of the Clock Drawing Test, there were no significant differences between the treatment groups.

In the subgroup of patients with pathologic results of the Clock Drawing Test, there was a marked decrease in the memory scanning time, measured as the slope of the reaction time curve, in the placebo group, and a marked increase in the memory scanning time in the selegiline group (MEDICATION × PERIOD, p = 0.047). In the subgroup of patients with normal results of the Clock Drawing test, no statistically significant differences between the treatment groups were observed.

In the subgroup of patients with pathologic results of the Clock Drawing Test, there was a slight increase in the total number of errors in the selegiline group, whereas there was a marked increase in the total number of errors in the placebo group (MEDICATION ×PERIOD, p = 0.029). No statistically significant difference between the treatment groups was observed in the subgroup of patients with normal results of the Clock Drawing Test.

The quantitative evaluation of the clock drawing pattern (Clock Drawing Scale) was conducted only at baseline and after 6 months. In the subgroup of patients with normal results of the Clock Drawing Test, the patients in the selegiline group showed better results on the Clock Drawing Scale than those in the placebo group (MEDICATION × PERIOD, p = 0.001), owing to a slight decrease in pathology in the selegiline group and a marked increase in pathology in the placebo group (Table 5). In the subgroup of patients with pathologic results of the Clock Drawing Test, there were no differences between the 2 treatment groups. The score reached almost its ceiling at baseline, preventing measurement of worsening.

As mentioned earlier, the Mini Mental State (MMS) Examination was given at baseline and after 24 weeks. ANOVA of the whole sample revealed a greater therapeutic effect in the selegiline group than in the placebo group in the Orientation-Place subscale (MEDICATION × PERIOD, $F[df\,1, 124]$ = 8.54, p = 0.004, Table 6). Additional analyses of the Clock Drawing Test subgroups revealed that in the subgroup with normal results, selegiline was superior to placebo in the MMS Orientation-Place subscale (MEDICATION × PERIOD, p = 0.041), whereas in the subgroup with pathologic results, the difference did not reach statistical significance.

The total score on the MMS examination also im-

Table 5: Results of the Clock Drawing Scale (see text for explanation)

			Score (mean and SD)		ANOVA						
						Normal results of CDT			Pathologic results of CDT		
Group	Subgroup by result of CDT	No. of patients	Baseline	Week 24	Variable	Degrees of freedom	F	p value	Degrees of freedom	F	p value
Placebo	Normal	28	1.8 (0.7)	2.7 (1.3)	MEDICATION	1, 50	1.25	0.269	1, 80	0.56	0.458
	Pathologic	37	4.1 (1.0)	3.3 (1.3)							
	Total	65	3.1 (1.4)	3.0 (1.3)	PERIOD	1, 50	5.94	0.018	1,80	16.63	0.001
Selegiline	Normal	24	2.1 (0.7)	1.9 (1.1)							
	Pathologic	45	3.7 (0.9)	3.3 (1.3)	MEDICATION × PERIOD	1, 50	12.27	0.001	1, 80	1.27	0.263
	Total	69	3.2 (1.2)	2.8 (1.4)							

Table 6: Results of the Mini Mental State Examination, Orientation-Place subscale (see text for explanation)

			Score (mean and SD)		ANOVA						
						Normal results of CDT			Pathologic results of CDT		
Group	Subgroup by result of CDT	No. of patients	Baseline	Week 24	Variable	Degrees of freedom	F	p value	Degrees of freedom	F	p value
Placebo	Normal	25	3.8 (1.1)	3.6 (1.4)	MEDICATION	1, 45	0.42	0.518	1, 79	0.99	0.324
	Pathologic	38	3.9 (1.1)	3.7 (1.3)							
	Total	63	3.9 (1.1)	3.7 (1.4)	PERIOD	1, 45	1.92	0.173	1, 79	0.22	0.637
Selegiline	Normal	22	3.5 (0.8)	4.2 (1.1)							
	Pathologic	43	3.4 (1.3)	3.8 (1.1)	MEDICATION × PERIOD	1, 45	4.44	0.041	1, 79	3.66	0.059
	Total	65	3.4 (1.1)	3.9 (1.1)							

proved significantly following selegiline treatment compared with placebo in the subgroup of patients with normal results of the Clock Drawing Test (MEDICATION × PERIOD, $F[df\ 1, 45] = 4.78$, $p = 0.034$), whereas in the subgroup of patients with pathologic results, no significant treatment effects were found.

In electroencephalograms (EEG) obtained at baseline and 24 hours later, the dominant frequency in the subgroup of patients with normal results of the Clock Drawing Test was faster than that in the subgroup of patients with pathologic results (CLOCK, $F[df\ 1, 100] = 8.86$, $p = 0.004$, Table 7).

In the subgroup of patients with pathologic results, there was a modest decrease in the dominant EEG frequency in the selegiline group and a profound decrease in the placebo group (MEDICATION × PERIOD, $p = 0.019$) after 6 months of treatment. No significant differences between the treatment groups were found in the subgroup of patients with normal results.

In terms of adverse events, the SARS scale did not reveal any significant differences in the adverse events profile between the selegiline and placebo groups.

There were no signficant differences between the treatment groups by centre.

Discussion

The main finding of our trial is that long-term treatment with selegiline, in comparison with placebo, improves cognitive and behavioural functions in patients suffering from mild to moderate Alzheimer's disease. The improvement is particularly pronounced in object and spatial memory, evaluated by Sternberg's Memory Scanning test and the Clock Drawing Scale, respectively. The prefrontal areas are essential for the maintenance of these memory functions, and, since they are rich in dopamine receptors,[34,35] they might be the target of the selegiline action. The observed improvement in the MMS Orientation-Place subscale may have the same basis. Another important area of improvement was sensorimotor speed, reflected by the intercept in Sternberg's Memory Scanning test.

At a different level of observation, the EEG findings indicate that selegiline may partially prevent age-related electrophysiological changes, which also means that the effect of selegiline may be quite robust, influencing basic biologic processes of central nervous system functioning.

The paradoxical prolongation of the memory scanning time in the selegiline group, reflected as an increase in the slope in Sternberg's Memory Scanning test, can be explained by a significantly higher number of errors in the placebo group. The increase in the error rate indicates that fewer memory traces were retained, with a consequent reduction in the memory scanning time. Hence, the increase in the reaction time slope following treatment with selegiline indicates an expansion of the memory storage capacity.

Another main finding of our study is the differential effect of selegiline in patients in different Clock Drawing Test subgroups. This is probably related to the psychometric properties of assessment instruments: the sensitivity to change of a psychometric test depends on the general degree of dementia; hence, the treatment effects in different stages of the disease may be reflected by different tests depending on their "item characteristic curve."[22] The Clock Drawing Test seems to have served as a useful tool for determining the disease stage, particularly in the context of this trial, since it reflects the function of the prefrontal visual associative areas, where dopamine plays an important role as neurotransmitter.[34,35] The validity the Clock Drawing

Table 7: Dominant electroencephalogram frequency changes (see text for explanation)

					ANOVA						
			Frequency, Hz (mean and SD)			Normal results of CDT			Pathologic results of CDT		
Group	Subgroup by result of CDT	No. of patients	Baseline	Week 24	Variable	Degrees of freedom	F	p value	Degrees of freedom	F	p value
Placebo	Normal	21	9,2 (1.3)	9.1 (1.5)	MEDICATION	1, 39	0.44	0.512	1, 61	0.10	0.754
	Pathologic	29	8.8 (1.2)	8.4 (1.4)							
	Total	50	9.0 (1.2)	8.7 (1.5)	PERIOD	1, 39	2.68	0.110	1, 61	2.16	0.147
Selegiline	Normal	20	9.6 (1.7)	9.3 (1.7)							
	Pathologic	34	8.5 (0.9)	8.6 (0.9)	MEDICATION × PERIOD	1, 39	0.93	0.342	1, 61	5,83	0.019
	Total	54	8.9 (1.4)	8.9 (1.3)							

Test as a measure of severity of dementia is supported by the EEG results (significant effect of CLOCK in ANOVA), which showed a lower dominant EEG frequency in the subgroup of patients with pathologic results of the Clock Drawing Test.

As far as we know, this is the first time the principle of differential data evaluation with regard to disease stage has been used to demonstrate the efficacy of a cognition-enhancing drug. We know from unpublished results of this trial that when other cut-points (e.g., a threshold score on the MMS) are used to break down the sample, the effect is similar to using the Clock Drawing Test. Hence, we may speculate that re-analyzing previous studies of cognition-enhancing agents using this principle could disclose significant treatment effects that remained obscured in traditional statistical analysis.

The exact mechanism of the action of selegiline in patients with Alzheimer's disease is still to be determined. For example, the 6 to 12 weeks' delay before improvement was seen in Sternberg's Memory Scanning tests excludes the straightforward interpretation that the immediate increased availability of dopamine is the main underlying mechanism. If this were the case, the improvement would have occurred much sooner. Therefore, our results support the idea that a neuronal rescue mechanism or antioxidant protective mechanisms or both play a role in the action of selegiline, because they involve long-term changes at the cellular level.

On the other hand, the direct dopaminomimetic effect of selegiline may be responsible for the increase in irritability in the patients treated with selegiline, detected by the NOSIE scale after 6 weeks of therapy, as well as for the occurrence of uncooperativeness or restlessness, which were the most frequent reasons for early withdrawal in the selegiline group. Adverse effects of this kind may have been amenable to control by decreasing the dose of selegiline in the affected patients.

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